Data sheet

Protein A SepFast™ MAG
Protein G SepFast™ MAG

Protein A SepFast MAG and Protein G SepFast are magnetic beads designed for coupling of antibodies enabling enrichment of target protein for further downstream analyses such as mass spectrometry (MS and LC-MS) and electrophoresis techniques. The base matrix is made of cross-linked agarose encapsulating magnetic material. Therefore, the resin possesses magnetic properties. Removal of liquid after each step such as binding, washing and elution can be readily done by fixing the resin with a magnet.

Our product can be used with any magnetic device commonly available in the market place or magnets of any shape.

Protein A and Protein G binds to the Fc region of immunoglobulins. The binding is highly specific so high purity can be achieved in a single step. The purification power of Protein-A ligand and Protein G ligand has been well documented in various antibody purification applications, such as isolation and purification of classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media. In general, protein G shows affinity to more classes of immunoglobulins than protein A (as illustrated in Table 1).

Table 1. Relative binding strengths for protein A and protein G

Species	Subclass	Protein A binding[1]	Protein G binding
Human	IgA	variable	-
	IgD	-	-
	IgE	-	-
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	-	++++
	IgG_4	++++	++++
	IgM	variable	-
Avian egg yolkIgY	-	-	
Cow		++	++++
Dog		++	+
Goat		-	++
Guinea pig	IgG_1	++++	++
Hamster		+	++
Horse		++	++++
Koala		-	+
Llama		-	+
Monkey (rhesus)		++++	++++
Mouse[2]	IgG_1	+	++++
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	++	+++
	IgM	variable	-
Pig		+++	+++
Rabbit	No distinction	++++	+++
Rat[3]	IgG_1	-	+
	IgG_{2a}	-	++++
	IgG_{2b}	-	++
	IgG_3	+	++

Sheep	+/-	++

[1] ++++ = strong binding; ++ = medium binding; - = weak or no binding
[2] IgG1 from mouse binds more strongly to protein G than to protein A.
[3] Note that IgG from rat binds to protein G coupled to SepFast.

1. Properties

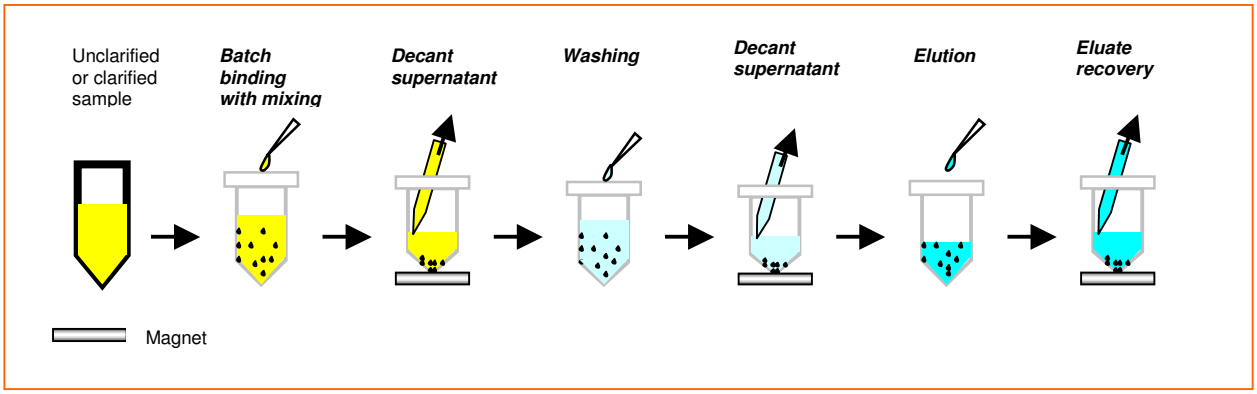
Protein A and protein G is immobilised to a highly porous and highly cross-linked magnetic agarose base matrix. Agarose has long been used for chromatographic separations due to its excellent hydrophilic and low non-specific-binding nature. The particles have an open pore structure with excellent mass transfer properties to large protein molecules. The medium shows high mechanical rigidity.

Protein A SepFast MAG and Protein G SepFast MAG is highly stable and compatible with a wide range of chemicals commonly required in antibody purification processes.

Table 2: Characteristics of Protein A SepFast MAG and Protein G SepFast MAG:

Matrix	Cross-linked agarose encapsulating magnetic material
Binding capacity	> 10 mg human IgG / ml gel
pH stability	2-10 (short term) and 5-9 (long term)
Working temperature	+4^{O}C to +30^{O}C
Chemical stability	Compatible with most commonly used reagents for antibody purifications
Sanitisation	Wash the resin with 2% hibitane/20% ethanol
Storage	20% ethanol at +4^{O}C - +8^{O}C

2. General operations



1. **Take out the magnetic bead.** Fully shake the bottle to re-slurry the resin. 25 µl slurry gives 5 µl settled beads. Take out the required amount of slurry using a 200 µl pipette (Note: the pipette tip should be cut off approx. 3-5 mm from the narrow side to avoid possible tip blockage by the resin particles) and transfer to a suitable container (e.g. an eppendorf centrifuge tube). Settle the beads with a magnet and remove the liquid.

2. **Equilibration.** Add 500 µl of the equilibration / binding buffer and briefly mix with the beads. After settling the particles using a magnet, remove the supernatant using a pipette.

3. **Antibody binding.** Add the antibody sample. Close the container lid and place in a suitable mixer (allowing up-side-down mixing) for batch binding. Depending on the nature and size of the target protein, the binding time varies from a few minutes to up to a few hours. Generally speaking, 10 – 30 minutes is sufficient to utilise most of the resin capacity.

4. **Washing.** Add 500 μl of the equilibration / binding buffer and briefly mix with the beads. After settling the particles using a magnet, remove the supernatant using a pipette.

5. **Target protein binding.** Add the antibody sample. Close the container lid and place in a suitable mixer (allowing up-side-down mixing) for batch binding. Depending on the nature and size of the target protein, the binding time varies from a few minutes to up to a few hours. Generally speaking, 10 – 60 minutes is sufficient. Afterwards, remove and collect the supernatant to a suitable tube (lable as un-bound protein).

6. **Washing.** Add 200 μl washing buffer (if the bead volume is less than 20 μl). Shake to fully mix with the beads. Settle the beads with a magnet. Collect the washing liquid to another tube. Repeat this washing step 2 more times.

7. **Elution.** Generally speaking, the eluent volume at 5 – 10 times the resin volume is sufficient to recover the bound protein. For the best recovery yield, incubating the resin-eluent mixture in a mixer for 5 - 10 minutes is recommended. This allows sufficient time for the internally bound protein molecules to diffuse out of the macropores. The eluate is recovered through pipetting after the particles are dragged down with a magnet. This step is repeated one more time to maximise the recovery yield. Combine the eluent together.

3. General considerations

3.1 Sample pretreatment

Clarifying of sample might be needed before applying it to the beads. To prevent target protein degradation, inhibition of protease activity may be required.

3.2 Binding

Protein A and Protein G SepFast MAG binds IgG from most species at neutral pH (e.g. pH 7 to 7.4) and physiological ionic strength (e.g. phosphate saline buffer). The typical binding buffer is 50 mM Tris/HCl + 150 mM NaCl, pH 7.5 (TBS buffer). Alternatively, 1.2 M K_2HPO_4 pH 9.0 should increase the affinity of mouse IgG1 to protein A. Washing buffer can be TBS, TBS + 1% octylglucoside or 0.1 M triethanolamine + 0.5 M NaCl, pH 9.0.

The static binding capacity depends on the source of the particular immunoglobulin.

3.3 Elution

The bound immunoglobulin is normally eluted by reduced pH, such as about pH 3.0. The general elution buffer includes 0.1M glycine pH 2.5 to 3.1, 0.1M citric acid pH 2.5 to 3.1, 2.5% acetic aicd, 2% SDS, 0.1 M ammonium hydroxide pH 10 to 11, 0.1 M phenyl phosphate etc. For very strongly bound molecules, the pH may reduce to 2.

For acid labile proteins, the eluted fractions can be quickly neutralized by adding (or pre-added) 1M Tris/HCl, pH 9.0 (10% to 20% v/v).

3.4 Other tips

In case of larger volume of starting material (> 1.5 ml), a 10 ml to 50 ml plastic tube could be used when binding the target protein. The beads can be settled by a magnet. Another alternative is to spin down the beads by using a swing-out centrifuge.

It could be advisable to transfer the magnetic bead solution to a fresh Eppendorf tube during the last wash buffer step. This action prevents potential elution of proteins non-specifically bound to the plastic material in the Eppendorf tube.

After elution, place the fractions in the freeze or add sample buffer if SDS-PAGE is to be performed to prevent sample degradation.

4. Storage

Store the loose medium in the presence of 20% ethanol at 4-8°C. Never freeze the medium.

5. Further information

Visit www.biotoolomics.com for further information or contact the technical team or sales representatives.

6. Ordering information

Product	Quantity*	Code no.
Protein A SepFast MAG	500 µl	280101
	5 ml	280102
Protein G SepFast MAG	500 µl	280201
	5 ml	280202

Other quantities or bulk volumes available on request

BioToolomics Ltd
Unit 30A, Number 1 Industrial Estate
Consett
County Durham, DH8 6TJ
United Kingdom

www.biotoolomics.com